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Comparative haematology of pre and post spawning common carp (*Cyprinus carpio*)

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Abstract

The present study was carried out for a period of 90 days (02 February to 02 May, 2017) with a view to investigate comparative haematological changes in *Cyprinus carpio communis* in relation to pre spawning and post spawning phases. Experimental breeding in common carp was performed for this haematological study. The haematological parameters such as Total Erythrocyte Count (TEC), Total Leucocytes Count (TLC), Haemoglobin (Hb), Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) of the experimental fish were compared in relation to sex and their reproductive status. The results clearly indicated that the blood parameter levels such as TEC, MCV, MCH and MCHC ($P < 0.05$) were highly significant and statistically significant Hb ($P < 0.05$) was observed during pre-spawning period in both the sexes while differences in TLC and PCV ($P > 0.05$) were statistically non-significant between males and females. During the post spawning period the TEC, PCV, MCV, MCH and MCHC ($P < 0.05$) were statistically highly significant whereas differences in TLC and Hb, ($P > 0.05$) were statistically non-significant in both the sexes.

Keywords: Hematological parameters, *Cyprinus carpio communis*, Sex, Pre and post spawners, Reproductive status

1. Introduction

Fish Blood is a fluid tissue within the cardiovascular system comprising plasma, erythrocytes, leukocytes, thrombocytes. It is one of the most active components that contribute to fish metabolic processes by ensuring gas exchange within the body and between the fish and the exterior environment. Any dysfunction of blood can have serious effects on the physiological activities of fish thereby resulting in pathological problems. The primary role of blood in fishes is oxygenation and nutrition of tissues, maintenance of acid-base balance and elimination of metabolic waste products from the body tissues. Thus, the assessment of haematological techniques proved valuable for fishery biologists in assessing the status of fish health.

Haematological studies have generally been used as an effective and sensitive index to monitor physiological changes in fishes, as these can provide valuable information about response of external environment on the internal physiology of fish. Further, haematological parameters are also frequently used as a tool to assess the health condition of fishes necessitated by the importance of pisciculture for fish production. Being a cold blooded vertebrate, the seasonal effect of the environment can change the physiology and biochemistry of blood in fishes.

Understanding the haematological characteristics is an important tool that can be used as an effective and sensitive index to monitor physiological and pathological changes in fishes. Normal ranges for various blood parameters in fish have been established by different investigators in fish physiology and pathology [1]. Blood parameters are considered useful criteria for indicating physiological disturbances in intensively farmed fishes and therefore, may provide useful information for prognosis and diagnosis of ailments in fish. Hematological indices have also been reported to detect physiological changes in fish, as well as indirectly assisting in monitoring the aquatic ecosystems [2]. Variations in blood indices may be related to natural physiological cycles, environmental stimuli, or both [3]. From this point of view, adequate knowledge about erythrocytes is the major and reliable indicator of various sources of stress [4]. Moreover, red blood cells can be used to identify and assess conditions that cause stress to the fish, and consequent ailments in the fish [5, 6]. These changes may be directly or indirectly affected by natural oscillations in environment [7], season [8, 9] feeding behavior [10]

and stress ^[11].

Therefore, it may be useful to build up an information repository of the baseline data of haematological status and related variations in different fish species in relation to their ambient environmental and physiological conditions. Many haematological studies have been conducted so far on various immunological aspects of different culturable fish species namely *Channa* ^[12, 13], *Tilapia* ^[14] and *Salmon* ^[15]. In this context, serological observations have been noticed as the most informative parameters to assess physiological status of a fish in relation to environmental conditions, species, sex and spawning activities.

2. Materials and Methods

2.1 Experimental Fish

For this study *Cyprinus carpio communis* was used as an experimental fish. The stock of *Cyprinus carpio communis* were collected from Aquaculture Research and Seed Unit of Directorate of Research, MPUAT, Udaipur.

2.2 Experimental Design

The present research was undertaken in the Aquaculture Research Unit, Directorate of Research, MPUAT, Udaipur, for a period of 90 days (February to May, 2017). For this purpose healthy and mature specimens of common carp were collected from fish rearing ponds using a drag net and subsequently the stock was segregated sex wise. These brood fishes were maintained in two cement tanks *viz.* Tank A and Tank B prior to their use for experimental breeding. For the spawning purpose sixteen fishes were selected and tagged (eight each males and females). During the experiment, the fish were fed @ 2% body weight on the diet comprising of rice bran and oil cake in the ratio of 1:1. The feed was offered to fish in submerged plastic trays.

2.3 Haematological analysis

2.3.1 Collection and analysis of blood samples

Each fish was individually caught by using scoop net and a sample was collected from caudal peduncle using a 2 ml syringe. Blood was collected in EDTA anti-coagulant vials.

The blood samples so collected were processed for the study of various haematological parameters such as Total Erythrocyte Count (RBC), Total Leucocyte Count (WBC), Packed Cell Volume (PCV), Haemoglobin (Hb), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), and Mean Corpuscular Haemoglobin Concentration (MCHC) in the laboratory using standard methods outlined in AOAC ^[16,17].

3. Results

The results of this study conducted on pre spawners and post spawners of the carp for different haematological parameters such as TEC, TLC, Hb, PCV, MCV, MCH and MCHC in the blood of the fish during February to May, 2017 have been presented in Tables 1.1 to 1.8. Possible correlations of the changes occurred in hematological parameters with respect to sex and spawning phases of the experimental fish have been summarized in these tables and explained herebelow:

3.1 Total Erythrocyte Count (TEC)

From Tabulated data it may be seen that the Total Erythrocyte Count in the blood of male fish during pre-spawning periods ranged from 1.13 to 2.10 x 10⁶/mm³ (Table-1.1) while in post spawning period it ranged from 1.10 to 1.46 x 10⁶ /mm³ (Table-1.3). In case of female fish the Total

Erythrocyte Count ranged from 2.13 to 2.64 x10⁶ /mm³ during pre-spawning period (Table-1.2) and 2.23 to 2.79 x10⁶ /mm³ during post spawning period (Table-1.4). The average Total Erythrocyte Counts in male and female fish blood were 1.47 x 10⁶ ± 0.30 and 2.34 x 10⁶ ± 0.19 /mm³ respectively, during pre-spawning period (Tables-1.1 and 1.2) while these values were 1.19 x 10⁶ ± 0.12 and 2.44 x 10⁶ ± 0.17 during the post spawning period (Tables-1.3 and 1.4).

3.2 Total Leukocyte Count (TLC)

Total Leukocyte Count varied between 7.36 to 9.10 x 10³/mm³ in male fish during the pre-spawning phase (Table-1.1) while in post spawning period, it ranged between 7.01 to 9.94 x 10³ /mm³ (Table-1.3). In the female spawner Total Leukocyte Count ranged from 6.95 to 8.30 x10³ /mm³ during pre-spawning period (Table-1.2) while the corresponding values ranged between 7.17 to 8.72 to x10³ /mm³ during post spawning period (Table-1.4). The average Total Leukocyte Counts in male and female fish blood were 7.63 x 10³ ± 0.60 and 7.63 x 10³ ± 0.49 /mm³ respectively, during pre-spawning phase (Tables-1.1 and 1.2) while the values were 8.06 x 10³ ± 0.97 and 8.14 x 10³ ± 0.53 during post spawning period, respectively (Tables-1.3 and 1.4).

3.3 Haemoglobin (Hb)

As evident from Table 1.1 the haemoglobin contents of *C. carpio* oscillated in male fish from 9.80 to 16.20 g % during pre-spawning period while in post spawning period, it ranged from 9.50 to 12.40 g % (Table-1.3). In the female fish, the value was within the range of 9.26 to 10.47 g % during pre-spawning period (Table-1.2) while in post spawning period it ranged from 8.75 to 10.47 g % (Table-1.4). Moreover, the average haemoglobin in male and female fish blood was 11.53±2.12 g % and 9.82 ± 0.41 g % respectively, during pre-spawning phase (Tables-1.1 and 1.2) while 10.23 ± 0.90 g % and 9.44 ± 0.60 g % during post spawning phase, respectively (Tables-1.3 and 1.4).

3.4 Packed Cell Volume (PCV)

Packed Cell Volume value in male fish varied from 24.10 to 40.10 % during pre-spawning period (Table-1.1) while in post spawning period, the indicated to vary from 16.40 to 32.40% (Table-1.3). In the female fish Packed Cell Volume value was within the range of 30.20 to 34.92% during pre-spawning period (Table-1.2) whereas in post spawning period it ranged from 26.80 to 32.92% (Table-1.4). The average Packed Cell Volume in male and female fish blood was 29.70±4.81% and 32.82 ± 1.76% respectively, during pre-spawning period (Tables-1.1 and 1.2) while these values were 21.40 ± 5.11% and 29.51 ± 2.45% during post spawning period, respectively (Tables-1.3 and 1.4).

3.5 Mean Corpuscular Volume (MCV)

The Mean Corpuscular Volume value in male fish varied from 190.95 to 239.82 fl during pre-spawning period (Table-1.1) while in post spawning period, it ranged from 149.09 to 221.91 fl (Table-1.3). As far as female fish is concerned, the Mean Corpuscular Volume value was within the range of 124.62 to 158.72 fl during pre-spawning period (Table-1.2). In post spawning period however, it ranged from 105.02 to 145.02 fl (Table-1.4). The average values of Mean Corpuscular Volume in male and female fish blood were 203.33 ± 15.66 fl and 141.32 ± 14.69 fl % respectively, during pre-spawning period (Tables-1.1 and 1.2) while the corresponding values were 178.37 ± 24.23 fl and 121.55 ±

14.63 fl during post spawning, respectively (Tables-1.3 and 1.4).

3.6 Mean Corpuscular Haemoglobin (MCH)

The Mean corpuscular haemoglobin value in male fish varied from 65.38 to 97.54 pg during pre-spawning period (Table-1.3) while in post spawning period, this value ranged from 83.33 to 88.59 pg (Table-1.5). In the female fish, mean corpuscular haemoglobin values were within the range of 39.65 to 45.68 pg during pre-spawning period (Table-1.4) whereas, in post spawning period it ranged from 31.36 to 46.12 pg (Table-1.6). The average mean corpuscular haemoglobin values in male and female fish blood were 79.15 ± 10.82 pg and 42.17 ± 2.57 pg respectively, during pre-spawning period (Tables-1.3 and 1.4). The corresponding average MCH values were 86.19 ± 2.04 pg and 38.93 ± 4.53 pg during post spawning period, respectively (Tables-1.5 and 1.6).

3.7 Mean corpuscular haemoglobin concentration (MCHC)

The Mean Corpuscular Haemoglobin Concentration value in male fish varied from 32.38 to 49.37% during pre-spawning period (Table-1.3) while in post spawning period, these values ranged from 38.27 to 57.92% (Table-1.5). In the female fish, Mean Corpuscular Haemoglobin Concentration was within the range of 27.10 to 31.80% during pre-spawning period (Table-1.4) whereas in post spawning period the same ranged from 29.86 to 34.05% (Table-1.6). The average Mean Corpuscular Haemoglobin Concentration in male and female fish blood was $39.06 \pm 5.78\%$ and $29.97 \pm 1.596\%$, respectively during pre-spawning period (Tables-1.3 and 1.4) while it was $49.15 \pm 7.12\%$ and $32.06 \pm 1.72\%$ during post spawning period (Tables-1.5 and 1.6).

3.8 Discussion

From the observations on various blood parameters made in the present study it is evident that the values of Total Erythrocyte Count (TEC), Total Leucocyte Count (TLC), and Mean Corpuscular Haemoglobin Concentration (MCHC) were higher during post spawning period in the female *C. carpio* which might be correlated with the relatively high metabolic rate during this reproductive phase and lower values during pre-spawning phase may be due to low metabolic rate. These findings are well supported by previous researches of [18, 19]. Further, water temperature fluctuations and day length are also reported to influence the haematology of fishes as documented in findings of [20, 21, 22].

In this study the Total Erythrocyte Count in the blood of female fish ranged from 2.13 to 2.64×10^6 /mm³ during a spawning period while 2.23 to 2.79×10^6 /mm³ during post spawning period. Likewise, total Leucocyte Count in the blood of female fish ranged from 6.95 to 8.30×10^3 /mm³ during pre-spawning period while such range was from 7.17 to 8.72×10^3 /mm³ during post spawning period. The Mean Corpuscular Haemoglobin Concentration value in the female fish was within the range of 27.10 to 31.80 % during pre-spawning period while in post spawning period it ranged from 29.86 to 34.05%. From these results it is obvious that in general, these blood parameters were higher during the post spawning phase as compared to pre spawning phase thus indicating higher metabolism in the post spawning phase.

The results of the present study further reveal certain blood parameters such as Haemoglobin (Hb), Packed Cell Volume

(PCV), Mean Corpuscular volume (MCV) and Mean Corpuscular Haemoglobin (MCH) were found lower in the post spawning period in the female *C. carpio*. These results corroborate with the findings of [23-31].

It is interesting to note that the Haemoglobin (Hb) and MCH values were higher in males than females during pre and post spawning periods. This might be due to higher physiological activeness of male than the female fish during this preparatory phase of breeding. These higher hematological values in male fish may be attributed to physiological activeness than the female fish as reported earlier by [32-34].

During study period the average Haemoglobin in male was higher than female fish in pre spawning period (Table 4.5 and 4.6). The variations were relatively higher range for Mean Corpuscular Volume in male fish during pre-spawning period (Table-4.3) whereas in post spawning period range was comparatively lower (Table-4.5). In the female fish, similar trends of variations were seen (Table-4.6). The average Mean Corpuscular Volume in male and female fish blood was higher during pre-spawning period (Tables-4.3 and 4.4) as compared to the post spawning period (Tables-4.5 and 4.6).

The Mean Corpuscular Haemoglobin Concentration value in male fish were lower in pre spawning period (Table-4.3) whereas in the post spawning period its range was higher (Table-4.5). In the female fish, Mean Corpuscular Haemoglobin Concentration value was within the range of 27.10 to 31.80% during pre-spawning period (Table-4.4) while in post spawning period it ranged from 29.86 to 34.05% (Table-4.6). The average Mean Corpuscular Haemoglobin Concentration in male and female fish blood was lower during pre-spawning period (Tables-4.3 and 4.4) while the same higher during post spawning period (Tables-4.5 and 4.6).

Considering the variations in blood parameters of two sexes, many workers established that Total Leucocyte Count in female fish is higher than male. The observed higher level of Mean Corpuscular Haemoglobin in male than their female counterparts could be explained by such differences. High levels of Mean Corpuscular Haemoglobin indicate more density of haemoglobin in a unit of Red Blood Cell [35]. From this point of view, the observed Total Leucocyte Count in female was significantly higher than the levels measured in the males, which indicate egg carriage, infection, adverse condition etc. as opined by [36].

Results of haematological parameters in this study clearly show that during pre-spawning period in both the sexes TEC, MCV, MCH and MCHC were highly significant. However, the differences between males and females for Hb was statistically significant but non-significant for TLC and PCV. During the post spawning period the differences in TEC, PCV, MCV, MCH and MCHC were statistically highly significant while for TLC and Hb, such differences were statistically non-significant in both the sexes. Some previous studies have however, reported no significant differences in blood parameters in *Prochilodus lineatus* [37] and in *Clarias gariepinus* [38], and *Barbus grypus* [39]. This can be accounted for specific mode of reproduction activities of particular fish species manifesting a different metabolic status.

On the basis of above observations therefore, it can be inferred that changes in various haematological parameters studied can be broadly correlated with reproductive activities performed by the common carp but without significant differences in male and female fish.

Table 1: Haemetological parameters of male *Cyprinus carpio* during pre-spawning period.

Parameters	TEC (x10 ⁶ /mm)	TLC (x10 ³ /mm)	Hb (g%)	PCV (%)	MCV (fl)	MCH (pg)	MCHC (%)
Minimum	1.13	7.36	9.80	24.10	177.90	77.10	36.10
Maximum	2.10	9.10	16.20	40.10	249.50	97.50	49.30
Mean	1.47	7.63	11.53	29.70	203.69	87.40	43.31
sd	±0.30	±0.60	±2.12	±4.81	±26.17	±6.51	±3.95

Table 2: Haemetological parameters of female *Cyprinus carpio* during pre-spawning period.

Dates	TEC (x10 ⁶ /mm)	TLC (x10 ³ /mm)	Hb (g%)	PCV (%)	MCV (fl)	MCH (pg)	MCHC (%)
minimum	2.13	6.95	9.26	30.20	182.00	81.25	36.26
maximum	2.64	8.30	10.47	34.92	195.40	94.43	48.25
mean	2.34	7.63	9.82	32.82	189.41	86.41	43.14
sd	±0.19	±0.49	±0.41	±1.76	±4.09	±5.04	±4.38

Table 3: Haemetological parameters of male *Cyprinus carpio* during post spawning period.

Parameters	TEC (x10 ⁶ /mm)	TLC (x10 ³ /mm)	Hb (g%)	PCV (%)	MCV (fl)	MCH (pg)	MCHC (%)
minimum	1.10	7.01	9.50	16.40	128.50	82.50	36.60
maximum	1.46	9.94	12.40	32.40	228.40	87.70	57.80
mean	1.19	8.06	10.23	21.40	172.49	84.83	42.79
sd	±0.12	±0.97	±0.90	±5.11	±45.33	±2.07	±8.81

Table 4: Haemetological parameters of female *Cyprinus carpio* during post spawning period:

Parameters	TEC (x10 ⁶ /mm)	TLC (x10 ³ /mm)	Hb (g%)	PCV (%)	MCV (fl)	MCH (pg)	MCHC (%)
minimum	2.23	7.17	8.75	26.80	160.20	78.25	38.25
maximum	2.79	8.72	10.47	32.92	189.76	90.20	52.14
mean	2.44	8.14	9.44	29.51	180.40	83.07	44.78
sd	±0.17	±0.53	±0.60	±2.45	±8.95	±4.61	±4.41

Table 5: Mean and SD of haematological parameters of male with respect to reproductive status.

S. No.	Parameters	Mean		SD	t-value
		Prespawning	Postspawning		
1	TEC	1.47	1.19	0.196	2.77*
2	TLC	7.63	8.06	0.651	1.09 NS
3	HB	11.53	10.23	1.412	1.72 NS
4	PCV	29.70	21.40	2.470	3.35*
5	MCV	203.69	172.49	58.548	1.75 NS
6	MCH	87.40	84.83	6.167	1.20 NS
7	MCHC	43.31	42.79	9.306	0.16 NS

* = significant

NS = Non significant

Table 6: Mean variations in haematological parameters of female with respect to reproductive status.

S. No.	Parameters	Mean		SD	t-value
		Prespawning	Postspawning		
1	TEC	2.34	2.44	0.063	1.16 NS
2	TLC	7.63	8.14	0.625	1.97 NS
3	HB	9.82	9.44	0.844	1.52 NS
4	PCV	32.82	29.51	1.160	3.15*
5	MCV	189.41	180.40	6.467	2.77*
6	MCH	86.41	83.07	1.460	1.38 NS
7	MCHC	43.14	44.78	2.560	0.75 NS

* = significant

NS = Non Significant

Table 7: Mean variation in haematological parameters of male and female with respect to sex during pre-spawning period.

S. No.	Parameters	Male		Female		Pooled sd	t-value
		Mean	s.d.	Mean	s.d.		
1	TEC	1.47	0.296	2.34	0.185	0.240	7.19**
2	TLC	7.63	0.597	7.63	0.494	0.546	0.01 NS
3	HB	11.53	2.116	9.82	0.406	1.261	2.71*
4	PCV	29.70	4.807	32.82	1.757	3.282	1.90 NS
5	MCV	203.69	26.171	189.41	4.088	15.129	1.89 NS
6	MCH	87.40	6.506	86.41	5.037	5.771	0.34 NS
7	MCHC	43.31	3.946	43.14	4.383	4.164	0.08 NS

* = significant ** = highly significant

NS = Non Significant

Table 8: Mean and SD for haematological parameters of male and female with respect to sex during post spawning period.

S. No.	Parameters	Male		Female		Pooled SD	t-value
		Mean	SD	Mean	SD		
1	TEC	1.19	0.116	2.44	0.174	0.145	17.25**
2	TLC	8.06	0.975	8.14	0.533	0.754	0.21 NS
3	HB	10.23	0.900	9.44	0.597	0.749	2.10NS
4	PCV	21.40	5.108	29.51	2.447	3.777	4.29**
5	MCV	172.49	45.325	180.40	8.947	27.136	0.58 NS
6	MCH	84.83	2.075	83.07	4.615	3.345	1.05 NS
7	MCHC	42.79	8.814	44.78	4.411	6.613	0.60 NS

**=highly significant

NS=Non Significant

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